The ability of the thromboelastogram (TEG® R-time difference between kaolin and heparinase) as a point of care test to predict residual heparin activity after in vitro protamine titration

Short title: TEG® [kaolin versus heparinase] R-time difference to predict heparin activity

Dissertation presented in partial fulfillment of the requirements for the degree of Masters of Medicine (Anesthesiology) in the Faculty of Health Sciences, University of Stellenbosch

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Declaration

I hereby declare that the content of this thesis is my own original work and that it has not previously been used in whole or in part in obtaining another degree or diploma.

Signature: Lauren Ann Joseph
Date: December 2017

Acknowledgements

I would like to thank Prof AI Levin for his guidance and help in this study especially regarding the design, preparation, statistical analysis and results.

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Chapter 1: Summary

Background: Differentiation between surgical bleeding and coagulopathy is critical as re-exploration is associated with increases in mortality and morbidity. Adequate reversal of heparin with protamine at the end of cardiopulmonary bypass (CPB) is critical to prevent postoperative bleeding. Meticulous dosing of protamine is required as excessive dosages has deleterious side effects on clotting. Traditional methods make use of an activated clotting time (ACT) for evaluation of adequate heparin reversal. However, recent use of other point of care (POC) tests, the thromboelastogram (TEG®) has started challenging the utility and exclusive use of ACT to evaluate effective reversal. Differences between thromboelastographic R-kaolin and R-heparinase times is an indicator of residual heparin. However, the exact relationship between these parameters and the exact amount of residual heparin is unknown. The rationale for this study was to accurately determine the relationship between the magnitude of the R-kaolin and R-heparinase time difference and blood heparin concentrations.

Aims: This study was performed to define the in-vitro relationship between the difference between the thromboelastographic R-kaolin and R-heparinase time difference (TEG® Delta-kh R-time) and plasma heparin concentrations. The primary outcome was to determined the relationship between the TEG® Delta-kh R-time difference and heparin concentrations. The secondary outcome was to determine the concentration of heparin at or below which R-kaolin times become measureable.

Methods:

This was a single centre, prospective, randomized laboratory study. Following institutional ethics approval and informed consent, sixty-two samples were taken during CPB from 20 patients meeting inclusion criteria. Samples were randomized to one of three groups which would dictate the protamine dose. The three groups were based on a protamine to heparin ratio (expressed as milligram protamine per milligram heparin administered to the patient) approximating 0.25, 0.5, and 0.75 mg/mg respectively. Each sample of blood was then administered a dose of protamine. The TEG® analysis entailed measuring the R-kaolin and R-heparinase time and noting the difference. Thereafter, each blood sample was sent for heparin concentration determination using an anti-Xa activity assay.

Results:

No relationship between the measurable R-kaolin time and heparin concentration could be demonstrated (p=0.80), as well as no relationship between measurable TEG® Delta-kh R- time difference and heparin activity (p=0.42). However, we did identify a high probability to be able to predict a measurable R-kaolin time (negative predictive value 90%, 95% CI 74% to 98%) when heparin concentration is less than 1.24IU/ml.

Conclusions:

We were unable to predict heparin concentration using TEG® in this study. It is likely that this was related to methodological problems. The protamine dose was a complex calculation and there is uncertainty with regard to the actual amounts used. There were also multiple laboratory technicians, with a possible loss of standardization.

However, R-kaolin time will likely be measurable at heparin concentrations below 1.24 IU/ml, and not measurable above that value. This observation is immensely valuable for clinicians and researchers. Future studies should take this into account and attempt to determine the relationship between TEG® Delta-kh R- time differences and heparin activity only when heparin concentration are less than 1.24IU/ml.

Opsomming

Agtergrond

Onderskeiding tussen chirurgiese bloeding en koagulopatie is krities belangrik, want hereksplorasie is geassosieer met ‘n toename in mortaliteit en morbiditeit. Die voldoende omkeuring van heparien met protamien aan die einde van Kardiolpulmonêre omleiding (KPO) is krities om postoperatiewe bloeding te voorkom. Noukeurige dosering van Protamien word benodig aangesien oormatige dosering nadelige newe-effekte op stolling. Geaktiveerde Sollingstyd (ACT) word gebruik om voldoende omkeuring van Heparien te evalueer. Onlangse gebruik van Point-of-Care toets, Tromboelastogram (TEG®), het egter die eksklusiewe gebruik van ACT uit te daag. ‘n Verskil tussen Tromboelastografiek R-kaolin en R-heparienase tyd is aanduidend van oorblywende heparien. Die presiese verhouding tussen hierdie twee parameters is nie bekend nie. Die rasionaal was om akkuraat die verhouding tussen die hoeveelheid van die verskil tussen die R-kaolin en R-heparienase tyd en bloed Heparien konsentrasies te bepaal.

Doel:

Hierdie studie was uitgevoer om die in-vitro verhouding te definieer tussen die verskil van Tromboelastografiek R-kaolin en R-heparienase tyd (TEG® Delta-kh R-tyd) en plasma heparien konsentrasies. Die primêre uitkoms was vasgestel as die verhouding tussen die TEG® Delta-kh R-tydsverskil en heparienaktiwiteits-konsentrasies. Die sekondere uitkoms was om die heparien konsentrasies te bepaal waaronder die R-kaolin tyd meetbaar raak.

Metodiek:

Hierdie was ‘n enkel sentrum, prospektiewe gerandomiseerde laboratorium studie. Na institusionele etiese goedkeuring en ingeligte toestemming, is 62 monsters geneem tydends KPO van 20 pasiente wat die insluitings kriteria vervul het. Die monsters was gerandomiseer tot een van drie groepe wat die Protamien titreringsdosering sal dikteer.

Die drie groepe was gebaseer op ‘n Protamien tot Heparien verhouding (uitgedruk as milligram protamien per milligram heparien toegedien aan die pasient) wat 0.25, 0.5,

en 0.75mg/mg onderskeidelik benader. ‘n Dosis Protamien was toegedien tot elke monster. Die TEG® analise het behels om die R-koalin en R-heparienase tyd te meet en die verskil daarvan aan te dui. Daarna is elke bloed monster gestuur vir die bepaling van die Heparien konsentrasie met die gebruik van ‘n anti-Xa aktiwiteitstoets.

Resultate:

Geen verwantskap tussen die R-kaolin tyd en Heparien konsentrasie kon getoon word nie. (P=0.80). Daar was ook geen verwantskap getoon tussen meetbare TEG® Delta-kh R- tyd en Heparien aktiwiteit nie. (P=0.42). Ons het wel ‘n hoë waarskynlikheid geidentifiseer om die meetbare R-kaolin tyd (negatiewe voospellings waarde 90%, 95% CI 74% tot 98%) te voorspel wanneer Heparien konsentrasie minder is as 1.24IU/ml.

Gevolgtrekking:

Ons was nie in staat om Heparien konsentrasie te voorspel, in die studie, met die gebruik van TEG® nie. Dit is moontlik dat dit toegeskryf kan word aan metodieke tekortkominge. Die Protamien titreringsdosering was ‘n kompleks berekening en daar is onsekerheid oor die werklike hoeveelhede wat gebruik was. Daar was ook veelvuldige laboratorium tegnikuste, ten spyte van opleiding, het ‘n moontlike verlies van standaardisering plaasgevind. R-kaolin tyd sal egter waarskynlik meetbaar wees by ‘n Heparien konsentrasie onder 1.24IU/ml en nie meetbaar by konsentrasies onder daardie waarde nie. Die observasie is van waarde vir klinikuste en navorsers. Toekomstige studies moet dit in oorweging bring en probeer om die verwantskap tussen TEG® Delta-kh R- tydsverskil en Heparien aktiwiteit te bepaal.

Chapter 2: Ethics Sumission

Protocol

Summary
References have been omitted from the summary.

Title: The ability of the thromboelastogram (TEG® R-time difference between kaolin and heparinase) as a point of care test to predict residual heparin activity after in vitro protamine titration.

Introduction, research question, hypothesis, aims and objectives:

The anticoagulant, heparin, is used to prevent fatal clotting while the patient is on the heart-lung machine during cardiac surgery. The heparin is neutralised using protamine, a hazardous drug. Inadequate dosages of protamine will leave the patient anticoagulated and can lead to excessive bleeding, while excessive dosages directly cause a coagulopathy. The test used to identify “adequate” reversal of heparin with protamine, activated clotting time (ACT), is a surprisingly inaccurate marker for heparin reversal. Regardless of this, clinical practice still largely makes use of this point-of-care (POC) test.

An alternative method POC test to determine if residual heparin is present and more protamine is needed is to use thromboelastography (TEG®). In particular, the difference in reaction time (R-time) between simultaneously performed ordinary TEG® and TEG® with heparinase (“heparinase TEG®”) has been confirmed recently as being a sensitive test of residual heparin. However, the exact relationship between these parameters has never been determined.

If this study was performed after protamine has been administered to the patient (the logical time), the results will likely be closely grouped and not provide sufficient scatter to determine the relationship between the two parameters. We therefore aim to perform the study using blood obtained from patients while on the heart- lung machine to which we will add a range of protamine dosages. These blood samples will have

both TEG® and heparin concentrations, the latter being the gold standard, determined.

The aim is to determine the exact relationship between the R time difference and heparin activity-concentration.

Null Hypothesis: The difference between the R-times of kaolin and heparinase TEG®, is not able to predict residual heparin activity after in vitro protamine titration. **Alternatively,** R-time difference is able to predict heparin activity.

A concise summary of the methodology:

If approval is granted and eligble patients grant consent, we wish to take a sample containing 10 millilitres (ml) of blood (this may be repeated two times: i.e. 3 samples of 10 ml of blood per patient) from patients who are heparinised on cardiopulmonary bypass (CPB). Protamine at effective doses of 0.25, 0.5 and 0.75 mg per 100 IU (1 mg) of heparin administered to the patient will be added to this blood sample. This sample will then be analysed by performing simultaneous kaolin and heparinase TEG®. We have clinical and research experience with TEG®. Similar quality control measures will be used as in previously published studies.

Samples to which protamine has been added will also be taken immediately to the coagulation laboratory, where they will be centrifuged and stored at -80 degrees Celsius. Heparin is stable at this temperature. Heparin levels will be measured in the coagulation laboratory of Tygerberg hospital using a modified anti-Xa chromogenic assay, the current gold standard method of heparin level determination.

The target population:

We will aim to enrol patients undergoing cardiac surgery using cardiopulmonary bypass at Tygerberg Hospital. Exclusion criteria will include all patients with prior known coagulation abnormalities except those having received unfractionated heparin and/or low dose aspirin preoperatively.

Anticipated benefits of the study:

This is novel research and no data on this relationship is currently available. The motive to undertake this research is to have a better understanding of the relationship of the kaolin-heparinase TEG® R- time difference and residual heparin concentrations. This will help us understand if this test is of use in identifing small residual amounts of heparin. This would be of use to prevent bleeding at the end of cardiopulmonary bypass.

The ethical considerations and risks for the patient:

1. There are no risks for the patient. The main risk would be of us performing the study incorrectly and/or making incorrect conclusions from the study.
2. The volume of blood removed from the patient is relatively small, the 30 ml (3 x 1o ml samples of blood), representing less than approximately 0,45% of the blood volume on cardiopulmonary bypass of a typical 70 kg patient. This should not affect transfusion requirements or anaemia postoperatively.
3. Even if small errors are made in the dose of protamine, this will not matter; it is not the dose of either protamine or heparin that matters but the relationship between the R time differences and the residual heparin concentrations that we wish to determine. Thus scatter of the data will be of value to determine the relationship.
4. Blood from patients on bypass will be used to perform this study: this ensures that any factors present during cardiopulmonary bypass that will affect the coagulation will be reflected by the TEG®. This study represents reality as closely as possible without changing practice. The heparin concentrations are also clinically relevant. This study could be performed by heparinising blood drawn form volunteers, then performing all the manipulations described above. However, whether this can be done in the requisite time (4 minutes) is not clear. The potential effects due to release of tissue factor VII and venous stasis during venepuncture is also eliminated with the study design chosen. The challenge of attempting to achieve typical heparinisation of blood is also eliminated when using this study design.
5. We have experience in this field having published before.
6. We have experienced technologists who perform TEG® to a high standard in a dedicated laboratory on a calibrated, well-maintained machine.
7. Our laboratory has previous experience in measuring heparin concentrations.

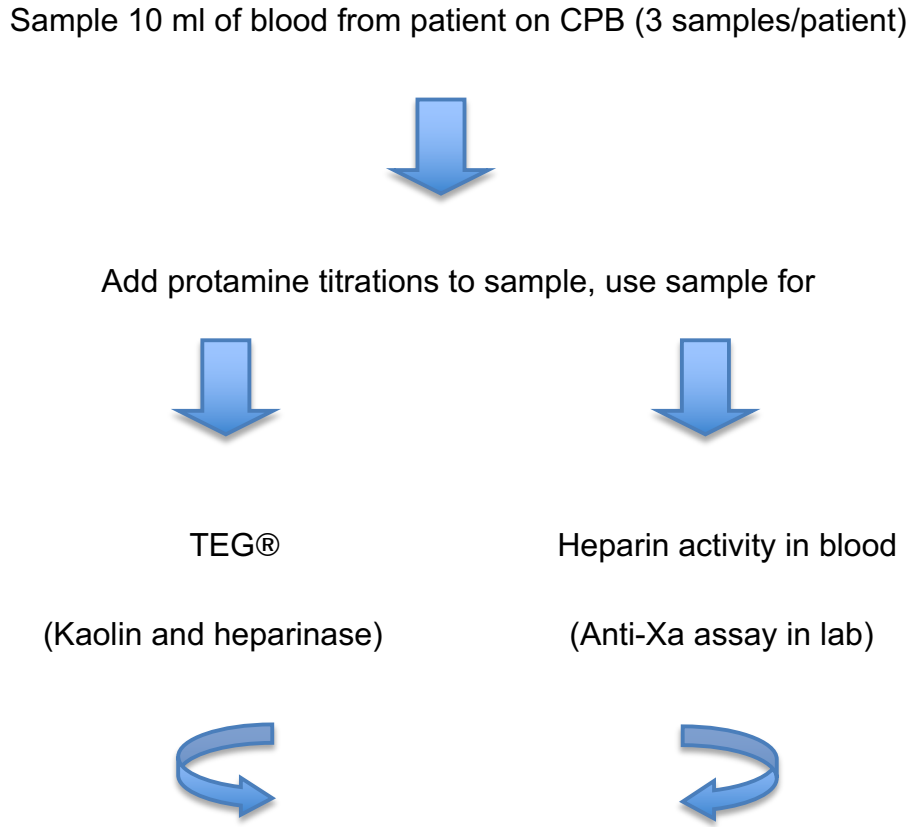
Subject population: Patients undergoing cardiac surgery at Tygerberg Hospital who will be heparinised while on cardiopulmonary bypass. Adults > 18 years. 20 patients (3 samples per patient) will be required. Therefore, a total of 60 samples will be taken (20 patients x 3 samples per patient).

Anticipated risks: Normal practice for patients undergoing cardiac surgery will be followed; this ensures representative samples will be collected. This study places no demands on the attending anaesthesiologist or changes in practice at all.

Anticipated benefits: The aim of this study is to equip doctors to more easily have a better endpoint of heparin reversal, which would imply better patient care with less risk involved for subsequent patients.

Final Ethical Considerations: There are no risks for the patient. Informed consent will be obtained. Patient autonomy will be respected. The study will take place in vitro and no new interventions are to be undertaken, and it will not influence the patient's management in any way. This research study will be submitted for approval by the Health Research Ethics Committee (HREC).

Diagram of protocol:



Literature review

Introduction

Postoperative bleeding is one of the main complications after surgery involving cardiopulmonary bypass (CPB); indeed this complication occurs in approximately 20% of patients.(1) Differentiation between surgical bleeding and coagulopathy is critical. Re-exploration for post-operative bleeding is associated with an increase in mortality and morbidity.(2) A surgical cause is found in 50% of patients; therefore many re-explorations can be avoided if a coagulopathy can be better identified and treated. These risks are further aggravated by the hazards, exorbitant cost and consequent intense scrutiny of the related transfusions that accompany postoperative bleeding.

It is therefore critical to monitor coagulation during and after surgery involving CPB. Laboratory tests are of little value during and after CPB, this being largely due to their delay in results. Their role in the pre-operative setting is still useful with the activated partial thromboplastin time (aPTT) being sensitive to low concentration levels of heparin. Various point of care (POC) tests such as activated clotting time (ACT), thromboelastogram (TEG®) and rotational thromboelastometry (ROTEM®) are available for this purpose. POC tests are defined as diagnostic tests at or near the bedside with the ability to produce rapid results.(3) This may provide guidance for the attending anaesthesiologist to correctly diagnose the cause of bleeding.(4) Coagulation monitoring with thromboelastography has been shown to decrease transfusion therapy and hence an improvement in outcome and diminished costs overall.(5, 6) Indeed, compared to TEG®/ROTEM® guided therapy, empiric therapy with blood and blood products are of questionable efficacy and may be hazardous to the patient.(7)

○ Residual heparin and inadequate protamine neutralisation
○ Heparin rebound phenomenon (reappearance of hypo-coaguability after adequate neutralisation of heparin with protamine)
○ Excess protamine (exhibits anti-platelet anticoagulant effects)
○ Hypothermia
○ Haemodilution
○ Dilution of clotting factors
○ Activation of the fibrinolytic system
○ Consumption or depletion of coagulation factors
○ Decreased or dysfunctional platelets
○ Surgical bleeding (50-70%).(1)

Table 1. Aetiologies for coagulopathy after CPB.(6)

○ Baseline activated clotting time test (ACT) from an arterial sample
○ Unfractionated heparin (200 -300 IU/kg) via central venous catheter
○ Repeat ACT after 3 minutes. Aim: 3–4 times of baseline ACT (480 seconds)
○ Initiate CPB
○ Unfractionated heparin (5000 IU) in CPB prime solution
○ Monitor ACT every 30 minutes during CPB
○ Maintain ACT 400–480 seconds during hypothermia (32- 34°C) while on CPB
○ Neutralise heparin with protamine after separation from CPB
○ Dose ratio 1-1,5mg protamine per 100 IU of heparin
○ Repeat ACT after 3–5 minutes
○ Aim: return of ACT to within 10% of baseline

Table 2: Standard anticoagulation management in our institution, TBH.

Apart from other causes of coagulopathy (Table 1), it is of particular importance to monitor adequate heparinisation and conversely, heparin neutralisation with protamine. Individual responses to a dose of heparin vary. Individuals may exhibit heparin resistance and require a higher dose due to acquired or inherited antithrombin III deficiency or increased protein binding of heparin.(8) At the end of surgery, it is critical to ensure that no residual heparin remains, potentially exposing the patient to the risk of developing a coagulopathy. This needs to be done in a timely manner and hence POC tests are ideal. It is however, of paramount importance to choose the correct POC test in order to ensure accuracy and meticulous protamine dosing. The following readily available POC tests, ACT, thromboelastogram (TEG®) and rotational thromboelastometry (ROTEM®) will now be discussed.

Activated Clotting Time (ACT)

Dr Paul G. Hattersley, an American pathologist, developed and subsequently validated the ACT test in 1966 as a test for diagnosis and management of patients with inherited coagulation disorders. Less than a decade later this whole blood coagulation assay had become a routine point of care test in surgery involving cardiopulmonary bypass.

The ACT test has been accepted as a rational and predictable measurement of the intrinsic coagulation process reflecting initial fibrin formation. The physical principle for this test is simple. Blood (2 ml) is placed into a glass tube containing a magnetic rod as well as an activator, the most common activators being kaolin or celite. The test tube is placed into a holder that simultaneously rotates and warms the test tube to 37,8 degrees Celsius. Resistance to movement of the steel rod in a magnetic field indicates clotting and is recorded by the timer. The normal ACT value ranges from 100-140 seconds and increases linearly with an increase in heparin concentration. Bull et al. set target values of 480 seconds as adequate heparin anticoagulation during CPB.(9, 10) This relationship is however distorted during hypothermia and haemodilution, thromboctyopaenia, impaired platelet function, different activators, or the use of aprotinin.(5, 11) All the aforementioned factors are unfortunately common during CPB; they independently affect ACT test results as follows:

- Hypothermia and haemodilution prolong ACT.(12)(17)

- Celite as an activator results in significantly longer ACT values than when kaolin is used.
- Aprotinin produces a dose-related prolongation of celite ACT, independent of heparin concentrations, this may lead to the sub-therapeutic heparin dosing.(13). Aprotinin has been withdrawn from the international market due to its association with increased risk of death compared to tranexamic acid or aminocaproic acid.(14)

Numerous iterations of the ACT device is available. Not all commercial ACT devices can be used interchangeably as different characteristics may exist. It is crucial to establish the instrument specific references values in a particular institution. For example, the Hemochron® system using a kaolin-based activator is a simple, widely used device for heparin management during and after CPB.(15) The MAX-ACT® is used in our institution, and may be used in conjunction with the Hemochron® instrument to improve accuracy according to the manufacturers. The Hepcon® device is a whole blood haemostatic system providing both ACT and accurate whole blood heparin concentration levels using automated protamine titration.(16)

ACT is relatively unreliable during the anticoagulated state, the very state we wish to monitor. Clinicians need to be aware of this and be cautious when making decisions regarding heparin neutralisation and protamine dosing based on ACT. In this respect, the return of the ACT to baseline is also not an absolute validation of complete heparin reversal as it appears that ACT is less sensitive to residual heparin than that of activated partial thromboplastin time (aPTT), TEG® or whole blood heparin assay.(5)

Dalbert et al. compared the Hemochron® ACT and Sonoclot® ACT devices. Despite having comparable accuracy, both were equally and significantly affected by haemodilution and aprotinin.(15) Despotis et al. demonstrated anticoagulation management during CPB with the Hepcon® device to be more reliable than the standard ACT device.(17) The great variability in the ACT results showed it to be insensitive to detect incomplete heparin reversal, inadequate to detect low levels of

heparin or the heparin rebound phenomenon. Reasons for this, once again are related to hypothermia and haemodilution so commonly experienced during CPB, as well as the presence of aprotinin or glycoprotein IIa/IIIb inhibitors. The poor correlation between ACT and plasma heparin concentration during CPB is also evident in infants <6 months. Guzetta et al. compared three commercially available ACT instruments to bedside and laboratory plasma heparin concentrations. They concluded that sole reliance of the ACT test is not advisable.(18) Galeone and colleagues also illustrated a poor correlation of ACT values to plasma heparin concentration, and hence, inability to detect residual heparin. In contrast, TEG® analysis showed significant association with plasma heparin concentrations, despite the exact relationship between the kaolin-heparinase R time difference not being published.(1)

Despotis evaluated the impact of heparin and protamine administration, guided by eiether ACT or ACT and whole blood heparin concentrations. While the protamine dose was similar in the two groups, the protamine: heparin ratio, the amounts of clotting factors and blood administered was less in the patients monitored with both ACT and heparin concentrations.

In summary, evidence shows that ACT may be a poor guide to heparin reversal for the attending anaesthesiologist. This may have risks with respect to post-operative bleeding.

Thromboelastogram (TEG®) and Thromboelstometry (ROTEM®)

The thromboelastogram (TEG®) and thromboelastometry (ROTEM®) are POC tests measuring whole blood visco-elastic properties allowing for more dynamic information regarding the coagulation process. Information regarding initiation and formation of the clot, the strength thereof, coagulation factor interaction and their interaction with platelets, platelet function and fibrinolysis is obtained within a short period of time. The R-time (reaction time) can be available within 4-8 minutes and further information regarding clot kinetics within 10-20 minutes. The rapid availability of such useful information is appealing during and after CPB. Laboratory tests (platelet count, prothrombin time, activated partial thromboplastin time and fibrinogen levels) are not only unable to provide information on clot kinetics, but also have a much greater delay between sampling and obtaining the results.(19)

Hartert introduced the concept of TEG in 1948, but it was only in 1996 that two different companies refined the method. TEG® is a registered trademark for the Haemoscope Corporation (USA) while ROTEM® is registered with Pentapharm GmbH (Germany). The physical principles of these two devices are comparable. The TEG® utilizes a pin attached to a torsion wire that is suspended in a blood sample in an oscillating cuvette. As the clot in the sample is formed, displacement of the pin occurs and is graphically depicted. ROTEM®, alternatively uses a stationary cup with the pin oscillating, transmission is via an optical sensor to a computer.(19)

Nomenclature in the quantitative information differs between the two devices but is comparable. Reference ranges however differ due to variations in cup size, material of cup and the different activators (Table 3). The attending anaesthesiologist must therefore be familiar with the device available in their institution.(19, 20)

Parameter	Units	Definition	TEG®	ROTEM®
Clotting time	s	Period from 0 to 2mm amplitude	Reaction Time R	Clotting time CT
Clot kinetics	s	Period from 2 to 22mm amplitude	Kinetics K Time	Clot formation CFT
Clot strengthening	deg	Slope between r and k/slope of tangent at 2mm amplitude	α	α
Amplitude Maximum strength Lysis	mm	Maximal amplitude	A Maximum amplitude MA CL 30, CL 60	A Maximum clot Firmness MCF LY 30, LY 60

Table 3. – TEG® and ROTEM® variables

Test	Activator/inhibitor	User/indication
Native	None	Non-activated assay
Kaolin	Kaolin	General coagulation assessment including platelet function
Heparinase	Kaolin and heparinase	Detection of heparin
Platelet mapping	Adenosine diphosphate arachidonic acid	Platelet function monitoring during anti-platelet therapy

Table 4. – Commercially available TEG® assays

Test	Activator/inhibitor	User/indication
na-TEM	None	Non-activated assay
ex-TEM	Tissue factor TF	Extrinsic pathway; fast assessment of clot formation and fibrinolysis
in-TEM	Contact activator	Intrinsic pathway; assessment of clot formation and fibrin polymerization
fib-TEM	Tissue factor and platelet antagonist	Fibrinogen level
ap-TEM	Tissue factor + Aprotinin	Fibrinolytic pathway; quick detection of fibrinolysis when combined with ex-TEM
Hep-TEM	Contract activator and heparinase	Detection of heparin
eca-TEM	Ecarin	Monitoring direct thrombin inhibitors (e.g., hirudin, argatroban)

Table 5. – Commercially available ROTEM® assays

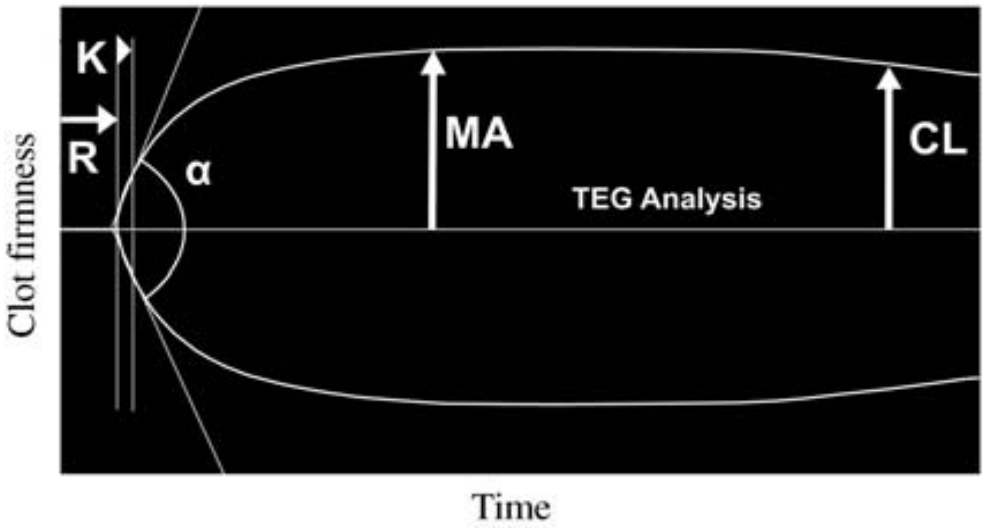


Figure 1.—Thrombelastography (TEG®) tracing. α: alpha angle; CL: clot lysis; K: kinetics; MA: maximum amplitude; R: reaction time.

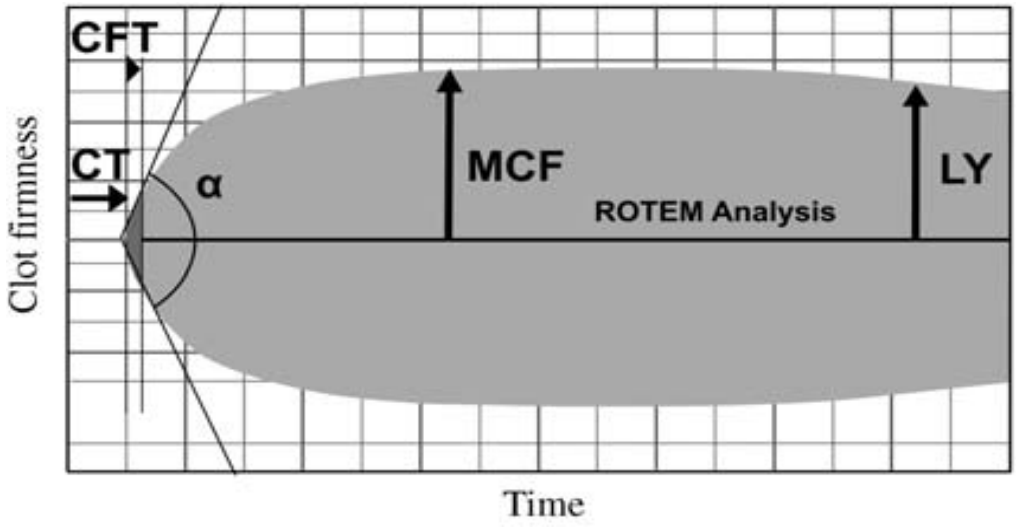


Figure 2.—Thrombelastometry (ROTEM®) tracing. α: alpha angle; CFT: clot formation time; CT: clotting time; LY: clot lysis; MCF: maximum clot firmness.

The R-time (reaction time) illustrates the time taken for initial clot formation. The effect of heparin in a sample will affect the R time on the TEG®. Other parameters, namely K-time, maximal amplitude (MA) and clot lysis remain unchanged.(1)

An enzyme obtained from *"flaviobacterium heparinum"* called heparinase, may be added to the cuvette. This specifically cleaves the polysaccharide portion of heparin, eliminating the effect of heparin in the sample. This allows for the detection of non-heparin haemostatic problems should they exist.(1) If the R-k time is prolonged, one would consider coagulation factor dysfunction; however, if this value has normalized after the addition of heparinase (R-heparinase time) the diagnosis of residual heparin can be made. Heparinase will not affect TEG variables of whole blood that do not contain heparin.(21) Nielsen, in an experiment using rabbits receiving stepwise heparin doses, found the TEG® to be more sensitive to changes in heparin activity than aPTT and ACT tests.(22)

Implementation of protocols guiding blood and blood product transfusions based on TEG® or ROTEM® have successfully demonstrated a decrease in requirements in adults and children undergoing cardiac surgery.(6, 23-25) It is of great importance for us that such an approach creates significantly greater cost efficiency. Furthermore, TEG® and ROTEM® share moderate agreement regarding indications for transfusion.(26) Shore-Lesserson et al. performed a randomized control trial. Comparing conventional to TEG®-based algorithms to guide blood and blood product transfusion management during CPB. Despite no significant difference in mediastinal tube drainage between the 2 groups, there was clinically significant less use of blood and blood product therapy in the TEG®-based group.(23). Royston and von Kier published similar results (Figure 3).(25)

Essell found the sensitivity of predicting blood loss to be similar between bleeding time, platelet count and TEG®, but TEG® to be the most specific. Furthermore they recommend that patients with an abnormal TEG® are at an increased risk of bleeding but that clinically excessive bleeding in the face of a normal TEG® implies surgical bleeding and that FFP and platelets should not be used empirically but attended to surgically.(27)

Subsequently, guidelines published in 2007 by the Society of Thoracic Surgeons and the Society of Cardiovascular Anaesthesiologists recommended the use of TEG® or ROTEM® to guide transfusion therapy.(28)

Limitations of the TEG®/ROTEM® include that trained personnel are required to perform the test. Correct device maintenance must be adhered to. Blood sampling and storage (native or citrated samples) may affect the test result. Native blood demonstrates less variability compared to citrated blood but should be used within 4-6 minutes. Citrated blood samples are stable for between 30 minutes to 2 hours for TEG® and for up to 6 hours for ROTEM®.(29, 30) Re-calcification before analysis is needed should citrated blood be used. Despite agreement with respect to identification of clotting and requirements for blood and factor usage, TEG® and ROTEM® parameters cannot be used interchangeably.(19) Finally, TEG® has never undergone all the validation procedures mandatory for conventional. Haemostatic tests such as intra- and inter-observer variability, repeatability, calibrations and quality controls.(31)

Despite the limitations, TEG®/ROTEM® does permit trace detection of residual heparin and can hence guide heparin reversal with protamine after CPB.(19) The available studies on the subject will now be reviewed.

Mittermayr challenged the routine use of ACT to confirm adequate reversal of heparin during CPB. Their institution, similarly to ours, uses a fixed dose of protamine calculated from the effective heparin dose. Adequate reversal thereof is then confirmed 15 minutes later with an ACT. Informed consent from 22 consecutive patients undergoing aorta-coronary bypass graft surgery was evaluated. Using the ROTEM® to detect adequate reversal rather than ACT. Their findings showed correlation of the ROTEM® (CT time) to heparin levels, regardless of haemodilution.

Galeone and colleagues similarly demonstrated that ACT, R-kaolin time TEG®, and the difference between R-kaolin and R-kaolin heparinase TEG® correlated well with plasma heparin concentrations. However, subsequent multivariate analysis indicated that only the TEG® tests correlated significantly with plasma heparin concentration

($p<0,05$) whereas ACT showed no correlation with plasma heparin concentration in both models.(1)

Recently, our institution published a single centre, blinded prospective study comparing two POC tests as endpoints of protamine titration during CPB surgery. For this purpose, 82 adult patients undergoing CPB with relevant exclusion criteria were randomized into a TEG and ACT management group. The hypothesis stated that heparinase kaolin thromboelastogram (TEG-HK) R time difference would reveal the need for additional protamine doses compared to if ACT alone was used. The results revealed no such difference and the hypothesis was rejected.(32) The study displayed good power and adequate quality control. The shortcomings were thus attributed to the following:

1) The initial dose of protamine to heparin ratio was relatively large (1,3:1 mg/mg).
2) The protamine dose was based on the initial effective dose of heparin and neglected possible metabolism thereof.
3) The endpoint of protamine titration in the ACT group was return to baseline ACT within 10%. However in the TEG group the range was not as strict and required a return to baseline within 20%. This had initially thought to be acceptable but later revealed a baseline of 10% should have been employed.
4) When comparing POC tests, the gold standard of plasma heparin concentration should be measured and compared accordingly.

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Protamine sulphate

Protamine sulphate, a derivative of salmon sperm, is used to neutralise the anticoagulant effect of heparin. Positively charged molecules form ionic complexes with the negative charges of heparin in a 1:1 ratio. The elimination half-life is 20-30 minutes. Side effects in the normal dose range (1-1,5 mg protamine per 100 IU heparin) include hypotension, decreased cardiac output, peripheral vasodilatation, bradycardia, potentially life-threatening pulmonary vasoconstriction and anaphylaxis. It has been demonstrated that protamine is toxic to the endothelium and the cardiac myocytes.(33). Proposed mechanisms to explain this include direct vasodilatation, depressed cardiac function,(34) histamine release and complement activation.(35)

Higher doses may contribute to bleeding during or post CPB.(36) Unbound protamine inhibits platelet activation, adhesion and aggregation.(37, 38) Ratios greater than 1,5:1 contributed to platelet dysfunction.(39) Furthermore, significant increase in the ACT is observed when ratios are above 2,6:1.(38)

The fixed dose ratio method used to calculate the dose of protamine ignores the proportion of the initial dose of heparin that may have been metabolized. This explains why ratios as low as 0,8:1 has been reported to provide adequate reversal. Reductions in these ratios have been shown to decrease postoperative bleeding as well as reduced blood and blood product transfusions.(17). Shigeta and colleagues used a titration method that adequately restored coagulation, preserved platelet response to thrombin and attenuated platelet alpha granule secretion during neutralisation**.** In their study, protamine doses in the titration group were less than half the doses in the fixed dose control group with no signs of heparin rebound or increased bleeding and hence adequate reversal.(40) This study therefore matched protamine administration to the amount of circulating heparin very effectively. The advantage to this method was a reduction in both protamine dose and potential toxicity. However, the clinician must be aware of a potential heparin rebound effect and subsequent bleeding.

Rationale

The use of POC tests has shown to reduce blood and blood product transfusion therapies (Figure 3). Therefore a goal directed algorithm guides the anaesthesiologist to make informed decisions. This may alleviate the deleterious effects of transfusion therapy as well as the cost burden of such therapies.(6) Empiric or prophylactic administration of transfusion therapy during CPB should not be entertained, neither the use of an insensitive or flawed monitor. Hence the POC test chosen to make such decisions should be as accurate as possible and comparable to the gold standard, in our proposed study, the focus will be on identifying the sensitivity of a particular POC test to heparin concentrations.

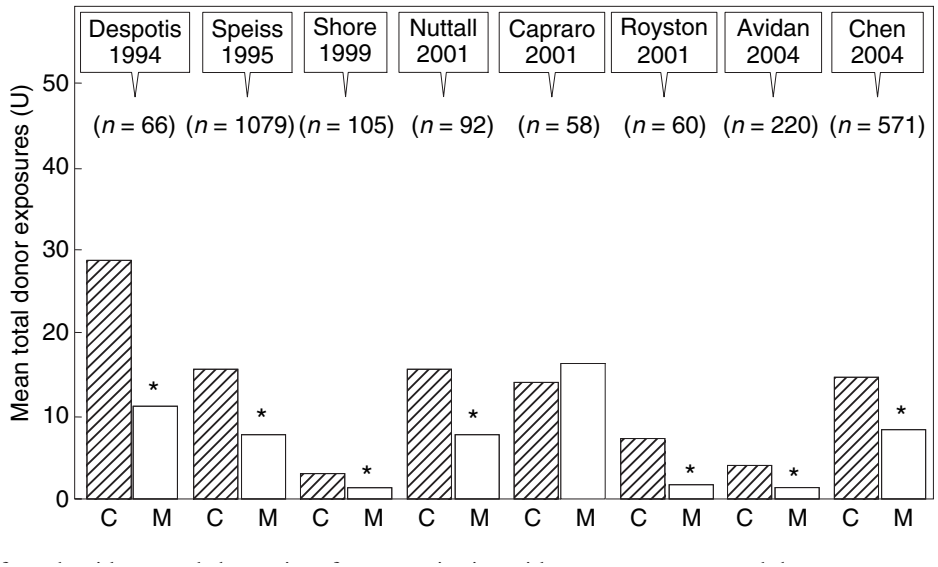


Figure 3. The impact of use of an algorithm coupled to POC monitoring with respect to mean total donor exposures observed in eight published studies. Hatched bar represents the mean total donor exposures within the control group (C) whereas the solid bar represents the mean total donor exposures within the group of patients who were treated with an algorithm coupled to point-of-care monitoring (M) perioperatively. Asterisks represent P < 0.05 between treatment cohorts. (41)

Every day Tygerberg hospital facilitates life saving surgery in the cardiothoracic theatres. A dedicated and highly specialized team of nurses and doctors focus on delivering the highest quality of medical and surgical care possible, making use of their knowledge as well as the resources available at their disposal. The attending anaesthesiologist makes critical decisions during and after cardio-pulmonary bypass, including the adequacy of the reversal of heparin. As previously mentioned, evidence indicates ACT to be an unreliable marker for heparin reversal. Regardless of this, clinical practice still largely makes use of this POC test. The TEG® has substantial evidence supporting the device as a POC test to guide and facilitate optimal decision making for coagulation monitoring. In this respect, a difference in R-time between a kaolin and heparinase TEG® implies that residual heparin is present in the blood. However, the exact relationship between the quantity of heparin that remains and the R time difference is not known. We wish to study the relationship between these variables. This is novel research and little if any precise data on this topic is currently available.

The motive to undertake this research is to better define the endpoint of reversal of heparin with protamine. This is important for many reasons. It may reduce postoperative coagulopathy with all the implications on blood and blood product usage and need for surgical re-exploration. Meticulous protamine dosing is critical to the patient and eliminates unwanted and harmful side effects.

Hypothesis

Null Hypothesis: The difference between the R-times of kaolin and heparinase TEG® is not able to predict residual heparin activity after in vitro protamine titration.

Alternate Hypothesis: The difference between the R-times of kaolin and heparinase TEG® is able to predict residual heparin activity after in vitro protamine titration.

Primary outcome

The relationship between the TEG® R time difference and heparin activity-concentrations.

Methods

Study design and target population

1. This will be a single centre, prospective, randomized, laboratory study.
2. The study population will consist of patients undergoing cardiac surgery at Tygerberg Hospital who will be heparinised while on cardiopulmonary bypass.
3. 20 patients will be required. 3 samples of 1o ml of blood will be taken per patient. Thus a total of 60 samples (20 patients x 3 samples) and a total of 30 ml of blood per patient (3 x 10 ml blood samples).

Inclusion criteria

Patients will be eligible for enrolment in this study if they meet the following criteria:

1. Elective or emergent cardiac surgery at Tygerberg Hospital.
2. Coronary artery bypass surgery or valve replacement procedures, or both combined.
3. On-pump coronary artery bypass surgery.
4. Patients who will be and are, heparinised for cardiopulmonary bypass.
5. Patients on low dose aspirin up to 150 mg daily including administration on the day of surgery.
6. Patients on intravenous heparin before surgery.
7. Adults exceeding 18 years of age and exceeding 45 kg body weight.

Exclusion criteria

Patients will not be eligible for enrolment in this study if they meet the following criteria:

1. Patients less than 18 years of age and less than 45kg in weight.
2. Patients scheduled to have off-pump surgery.
3. Pre-existing, known or suspected coagulopathy or administration of anticoagulants such as:
    3.1. Confirmed or suspected antithombin 3 deficiencies
    3.2. Low molecular weight heparin in the 48 hours prior to surgery
    3.3. Known protein S and/or protein C deficiency
    3.4. Other known pre-existing coagulation defects, acquired or congenital.
    3.5. Antiplatelet agents other than low dose cardiac aspirin in the last week before surgery

3.6. Anticoagulants other than heparin preoperatively in the last 2 weeks before surgery
3.7. Hepatic dysfunction affecting synthesis of coagulation factors with international normalised ratio exceeding 1.5
3.8. Renal dysfunction with urea or creatinine exceeding 15 mmol/l and 300 umol/l respectively.

Patient preparation

1. The normal preparation for a patient undergoing cardiac surgery will be followed. This study places no demands on the anaesthesiologist or changes in practice at all.
2. The anaesthetic technique will be left to the discretion of the anaesthesiologist.
3. The dose of heparin administered will also be left to the discretion of the anaesthesiologist and the perfusionist.

Data collection

The relevant data will be noted on a purposely-designed data collection form as specified in Appendix A. The following data will be collected:

1. Patient demographics; patient weight, length, gender, INR, PTT, platelet count, urea, creatinine, recent anticoagulants and procedure to be performed.
2. Haemoglobin concentration as measured by blood gas machine within the last 20 minutes
3. Volume of Balsol added to pump
4. R times of TEG-k and TEG-kh

Randomization

The samples will be randomized according to a scheme worked out on the website www.randomization.com for the following ratios of protamine: heparin ratios: 0.25, 0.5:1 and 0.75:1. (Appendix B)

Sampling and performance of TEG®

1. Each patient will have 3 samples of blood taken during CPB. Each sample will contain 10 ml of blood. Thus, a total of 30 ml per patient (3 x10 ml)
2. 10 ml of blood taken from CPB circuit arterial-venous reservoir bypass in a new 20 ml plastic syringe.
3. Protamine will be added, undiluted using an insulin syringe, to the 10 ml sample

containing the blood drawn via the bypass machine. The protamine dose will be randomised according to the scheme above. The exact protamine dose will be calculated according to patient weight, gender, and total heparin dose administered to the patient using a spreadsheet created specifically for this purpose. The calculations are as follows:

3.1. Estimated blood volume = blood volume (ml/kg) x body weight (kg) where estimated blood volume is considered to be 65 and 75 ml/kg for females and males respectively.

3.2. Protamine dose to be added to the 10 ml blood sample (mg) = (Protamine: heparin ratio (mg/mg) x total dose of heparin administered to patient (mg)) x (10 ml/estimated blood volume (ml)). The protamine: heparin ratio will be determined from the randomization scheme.

4. 1 ml of air will be drawn into the 10 ml blood sample containing the protamine and blood mixture, this syringe will be inverted 4 times to ensure complete mixing of the protamine and the blood.

5. Blood will be withdrawn from the 10 ml blood sample for performance of the kaolin and kaolin-heparinase TEG® by the technologists trained to perform this test using a similar standardised procedure. We will conduct a quality control to ensure that the two operators produce comparable TEG® results. Acceptable reproducibility is defined as being able to conduct 3 sets of tests with less than a 10% difference between parameters. The biweekly TEG® quality control will be meticulously performed.

6. The time frame for drawing of the blood, adding protamine, performing the TEG® and adding the sample to the citrated tube will be a maximum of four minutes.

7. The TEG® will be stopped after the R-times have developed and been noted. These R-times will be noted down on the data sheet, and a printout hereof will also be kept.

Determination of Heparin levels

1. 4,5 ml of the blood sample to which protamine has been added will be added to a labelled, citrated test tube for analysis of heparin activity.
2. The sample tubes will be placed on ice and immediately be taken to the coagulation laboratory, where they will be centrifuged and the plasma stored at -80 degrees

Celsius. Heparin is stable at this temperature. Heparin assay will be performed in batches. Heparin levels will be measured using a modified anti-Xa chromogenic assay, the current gold standard method of heparin level determination.

Data management

1. Intra operative data will be collected on a predetermined data sheet. (Appendix A) The data will be de-identified so that no data that can directly identify any patient.
2. The data will be entered into a Microsoft Excel® spreadsheet for processing and thereafter, statistical analysis.

Sample size

1. Prof Johan F Coetzee of the Department of Anaesthesiology and Critical Care has been consulted regarding study design, power and statistical analysis.
2. If a linear relationship is assumed between parameters, the regression equation will be one of a straight line, i.e. $y = bx + c$, where y is the dependent variable (heparin activity) and x is the independent variable (R time difference). Using the procedure for linear regression in the PASS software, the following result was obtained, assuming the following:
   2.1. Standard deviation of the x values 0, 0.5, 1.0, 1.5 (standard deviation 0.56) (i.e. using fractional values rather than percentages)
   2.2. That you would require a power of 0.95
   2.3. Two sided alpha 0.05
   2.4. A slope of at least 0.5
   2.5. A Pearson's product moment correlation coefficient (r) between x and y of at least 0.5.
3. In summary, a sample size calculation for linear regression revealed that a sample size of at least 42 samples is required to detect with 95% power the following relationship: A slope of 0.5 and a Pearson's product moment correlation coefficient of 0.5 with a two sided alpha value of 0.05 assuming a standard deviation of the R time differences of 56%.

3.1. As this is a pilot study, we wish to increase the sample size by approximately 50% to 60 samples to ensure that we do not incur a beta error should our sample size be estimations be somewhat incorrect.

Numeric Results for Two-Sided Testing of B = B0 where B0 = 0.00

Power	Sample Size (N)	Slope (B)	Standard Deviation of X (SX)	Correlation (R)	Standard Deviation of Residuals (S)	Alpha	Beta
0.95453	42	0.50	0.56	0.50	0.48	0.05000	
0.04547							

Statistical analysis

1. Data will be analysed using Medcalc® for Windows.
2. The data will first be analysed for normality of distribution and equality of variance using the Kolmogorov Smirnov and Levene Median tests respectively.
3. Parametric data that is normally distributed will be analysed using a two-tailed two-sample t test. Data will be presented as mean, standard deviation and 95% confidence interval of the difference between the means. Nonparametric data and data that is not normally distributed will be analysed using the Mann Whitney Rank Sum test. Data will be presented as median, 25 and 75th percentiles. Nominal and ordinal data will be analysed using Chi squared tests.
4. The relationships between the TEG® R-time difference and the heparin concentrations will be analysed depending on whether the data is normally distributed or not using Pearson or Spearman's rank coefficient tests. The mathematical relationship describing the best fit between the parameters will be determined using Excel®.
5. A p value of < 0.05 will be accepted to represents a statistically significant difference between parameters.

Ethical considerations

1. There are no risks for the patient. The main risk would be of us performing the study incorrectly and/or making incorrect conclusions from the study.
2. Blood sample volumes will comprise only 10 ml specimens at a time. 3 samples per patient.
3. Patients enrolled will be those who are scheduled to be administered heparin on cardiopulmonary bypass.
4. The population studied could be considered vulnerable, but we managed them ethically. Informed consent will be obtained.
5. The study population consists of patients undergoing elective cardiac surgery at Tygerberg Hospital
6. The study population will thus be fairly chosen, and will not be exposed to any additional risks.
7. From an ethical point of view, we are of the opinion that this study is sound. Patient autonomy will be respected through proper consent prior to enrolment of all participants. The study will take place in vitro and no new interventions are to be undertaken, and it will not influence the patient's management in any way. The aim of this study is to equip doctors to more easily have an endpoint of heparin reversal, which would imply better, and more scientific, patient care with less risk involved after the study.
8. This research study will be submitted for approval by the Health Research Ethics Committee (HREC) at the University of Stellenbosch and will be done according to internationally accepted ethical standards and guidelines.
9. Informed consent will be obtained from patients with the use of attached forms for this purpose. (See Appendix C)
10. A consecutive number will be assigned to each patient and data capture and presentation will be performed with these numbers. Patient privacy and confidentiality will thus be protected.
11. Participants have the right to withdraw from the study at any time.

Potential strengths of the study

1. There are no risks for the patient. The main risk would be of us performing the study incorrectly and/or making incorrect conclusions from the study.
2. The volume of blood removed from the patient is relatively small, the 30 ml representing less than approximately 0,45% of the blood volume on cardiopulmonary bypass of a typical 70 kg patient. This should not affect transfusion requirements or anaemia postoperatively.
3. Even if small errors are made in the dose of protamine, this will not matter; it is not the dose of either protamine or heparin that matters but the relationship between the TEG® R-time difference and the residual heparin concentrations that we wish to determine. Thus scatter of the data will be of value to determine the relationship.
4. Blood from patients on bypass will be used to perform this study: this ensures that any factors present during cardiopulmonary bypass that will affect the coagulation will be reflected by the TEG®. This study represents reality as closely as possible without changing practice. The heparin concentrations are also clinically relevant. This study could be performed by heparinising blood drawn form volunteers, then performing all the manipulations described above. However, whether this can be done in the requisite time (4 minutes) is not clear. The potential effects of release of tissue factor VII and venous stasis during venipuncture is also eliminated with the study design chosen. The difficulties of attempting to achieve typical heparinisation of blood are also eliminated when using this study design.
5. We have experience in this field having published before.
6. We have experienced technologists who perform TEG® to a high standard in a dedicated laboratory on a calibrated, well-maintained machine.
7. Our laboratory has previous experience in measuring heparin concentrations.
8. Envisaged outputs of this study include attainment of a Masters degree in Anaesthesiology and Fellowship in the College of Anaesthesiology (FCA) specialist degree. This aids student training. If at all suitable, it will be considered for publication.

Potential limitations of the study

1. We are not performing this study after administration of protamine to patients after bypass. While this would be ideal, we foresee that there would be significant

clumping of the data, making definition of the relationship between TEG® R-time difference and heparin concentrations difficult to define with accuracy.

2. We could perform this study by giving the protamine fractionally and then determining the relationship between TEG® R-time difference and heparin concentrations. This may have more risks for the patient as this alters clinical practice significantly, is not representative of clinical practice, puts the patient at risk of significant bleeding and would prolong surgery significantly.
3. It is an in vitro study that does not follow clinical practice exactly.

Timeframes

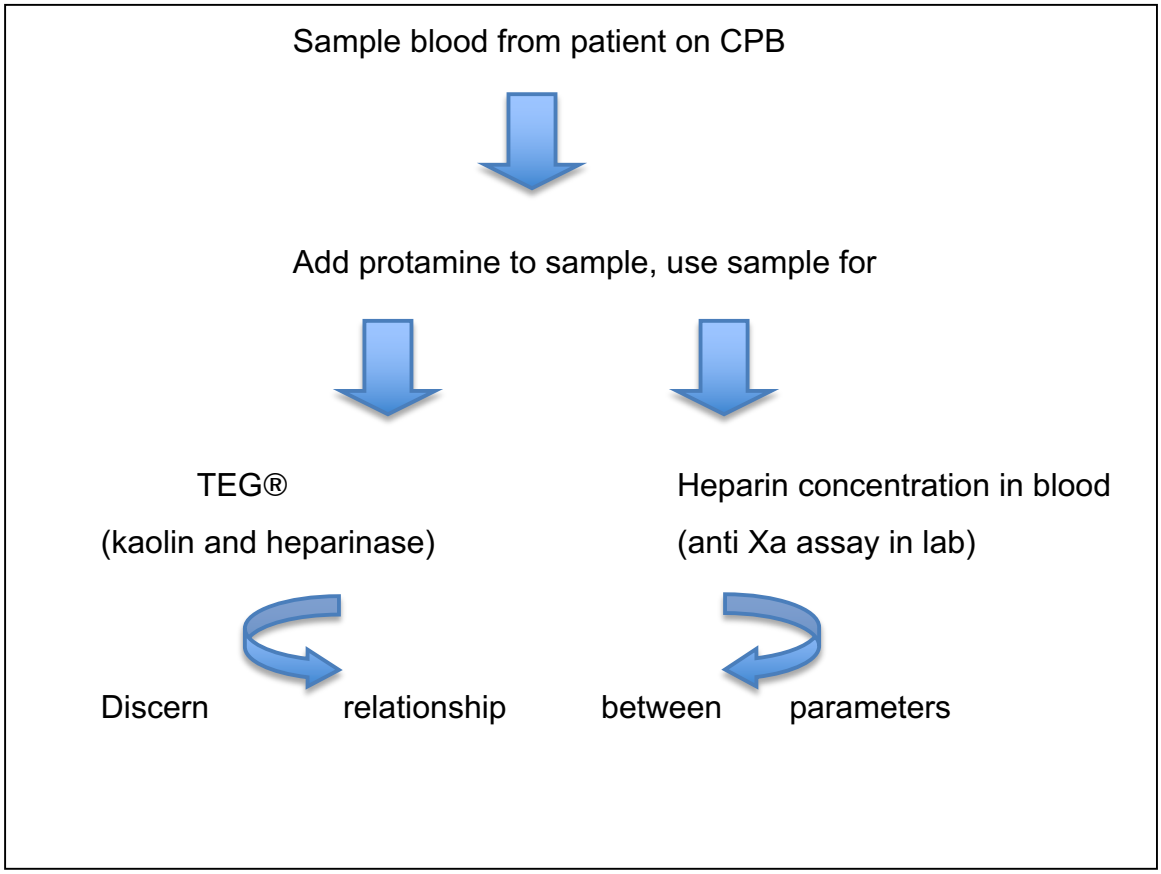
1. **Timeframe for collection of samples:** It is estimated that 3 patients per week will qualify for enrolment, and if a total of 20 patients are required, data collection should take between 6 to 8 weeks.
2. **Timeframe for sample processing:** The measurement of heparin concentrations will be processed as a batch. This will take approximately one to two months, depending on if this occurs over the December /January period.
3. **Processing of samples and data capturing**: 2 months
4. **Data analysis:** This will take 1 to 2 months
5. **Timeframe for writing up and discussing results:** 12 months. This is an important part of the process and we wish to be thorough in this regard; it also usually takes longer than expected.

Costs and funding

1. Application to the Harry Crossley Foundation for funds has been made and granted.
2. Cepheid will sponsor a second TEG® device for the duration of the study.

CONSUMABLES	
Syringes and needles:	R 200
TEG® consumables: R150 x 126	R18 900
Determination of heparin concentrations: R 600 x 63	R 37 800
Protamine: R 50 x 12 ampoules	R 600
Total costs	R 57 500

Summary of the protocol



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Appendix A: Data Collection form

TEG [Kaolin versus Heparinase] R-time difference to predict heparin activity

Date
Time
Sample number according to randomization
Protamine dose according to spread sheet calculator
Patient demographics
Weight
Length
Gender
INR preoperative
PTT
Platelet count preoperative
Urea preoperative
Creatinine preoperative
Preoperative anticoagulants
Procedure to be performed
Intraoperative data:
Heparin dose administered total intraoperatively
Hemoglobin concentration within the last 20 minutes
Most recent ACT within the last 20 minutes
Volume of Balsol added to pump
Coagulation data:
R time TEG-Heparinase
R time TEG-Kaolin

Appendix B: Randomization schedule

A Randomization Plan

from

http://www.randomization.com

```
 1.  0.5
 2.  0.75
 3.  0.5
 4.  0.25
 5.  0.5
 6.  0.5
 7.  0.25
 8.  0.5
 9.  0.75
10.  0.25
11.  0.25
12.  0.75
13.  0.75
14.  0.25
15.  0.75
16.  0.5
17.  0.25
18.  0.25
19.  0.75
20.  0.5
21.  0.75
22.  0.5
23.  0.75
24.  0.5
25.  0.75
26.  0.75
27.  0.5
28.  0.5
29.  0.25
30.  0.25
31.  0.75
32.  0.25
33.  0.75
34.  0.75
35.  0.25
36.  0.25
37.  0.25
38.  0.5
39.  0.5
40.  0.5
41.  0.25
42.  0.75
43.  0.5
44.  0.75
45.  0.5
46.  0.5
47.  0.75
48.  0.5
49.  0.5
50.  0.25
51.  0.25
52.  0.25
53.  0.5
54.  0.25
55.  0.25
56.  0.75
57.  0.75
```

```
58.  0.75
59.  0.75
60.  0.5
61.  0.75
62.  0.25
63.  0.25
```

63 subjects randomized into blocks of
21 21 21
To reproduce this plan, use the seed 21051
along with the number of subjects per block/number of blocks
and (case-sensitive) treatment labels as entered originally.
Randomization plan created on 24 October 2014 at 6:03:51 PM SAST

Appendix C: Informed consent

[Please note that we have excluded randomization from the informed consent, as it has no bearing on the patient].

Participant information leaflet and consent form

Title of the research project:

"The ability of the thromboelastogram (TEG® R-time difference between kaolin and heparinase) as a point of care test to predict residual heparin activity after in vitro protamine titration".

Reference number:

Principal investigators: Dr L Joseph, Prof AI Levin

Address: Department of Anesthesiology and Critical Care, University of Stellenbosch and Tygerberg Academic Hospital

Contact number: 021-9389230

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee (HREC) at Stellenbosch University and will be conducted according to the ethical guidelines and principles of the International Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

This study is about how good a test is to see the remaining amount of an anticlotting agent is after administration of an antidote.

Where will this research project take place?

It will take place in Tygerberg Hospital theatres. There will be approximately 20 participants.

What is the aim of this research project?

This experiment is designed to test how accurate the TEG® test is in predicting how much blood thinner (heparin) remains after surgery.

The information obtained from this experiment will help us treat future patients more accurately.

That is why we wish to do this study.

Explain all the procedures.

The procedures used during heart operations will be done as usual.

Once you are on the heart lung machine, small samples of your blood will be taken from the machine.

The amount of blood is two teaspoons (ten millilitres) at 3 separate times. You have approximately 650 times this amount of blood in your body and your body will not notice this amount of blood removal.

The experiments will be performed on your blood in test tubes outside your body. TEG® tests as well as direct measurement of blood thinner (heparin) will be done at the same time and the results compared. This will give us information about how accurate the TEG® test is.

The experiment will be performed in a test-tube outside your body and therefore, there is no risk to you.

Why have you been invited to participate?

You are invited to participate in this study because you are undergoing cardiac surgery.

What will your responsibilities be?

You will not have to do anything additional, nor will you receive any treatment other than the treatment you would have received if you are not participating in the study.

Will you benefit from taking part in this research?

You personally will not benefit from the study, however future patients may benefit from the results of the study.

Are there risks involved in your taking part in this research?

There are no additional known risks for your participation in this study, apart from the risks associated with the surgery itself as explained by the surgeon.

If you do not agree to take part, what alternatives do you have?

Whether you agree to participate in the study or not, you will receive the same treatment.

Who will have access to your medical records?

The information gained from the study is anonymous, and any information will be treated as confidential. If the information is used in a publication or thesis, your identity will not be revealed. The doctors conducting the study will have access to the information and will treat it as confidential. If anything of relevance to your health is noted, you will be informed thereof. The auditors or ethics committee members may need to inspect research records.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

As you are not receiving any treatment different from the standard care, there are no additional risks involved if you participate in the study.

Will you be paid to take part in this study and are there any costs involved?

No you will not be paid to take part in the study. There will be no costs involved for you, if you do take part. The Harry Crossley Fund has kindly funded the project.

Cepheid will also sponsor a second TEG® machine, allowing us to perform the laboratory tests.

Is there anything else that you should know or do?

You can contact Dr L Joseph at telephone 021-9385142 or Prof AI Levin at 021 9389230 if you have any further queries or encounter any problems.

You can contact the Health Research Ethics Committee at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.

You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled **"The ability of the thromboelastogram (TEG® R-time difference between kaolin and heparinase) as a point of care test to predict residual heparin activity after in vitro protamine titration".**

I declare that:

I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
I have had a chance to ask questions and all my questions have been adequately answered.

I understand that taking part in this study is voluntary and I have not been pressurised to take part.
I may choose to leave the study at any time and will not be penalised or prejudiced in any way.

Signed at (*place*) ....…….. on (*date*) ……...…….. 2015.

Signature of participant Signature of witness

Declaration by investigator

I *(name)* ……..…… declare that:

I explained the information in this document to ……..

I encouraged him/her to ask questions and took adequate time to answer them.

I am satisfied that he/she adequately understands all aspects of the research, as discussed above.

I did/did not use a translator. (If a translator is used then the translator must sign the declaration below.

Signed at (*place*) .....……..…….. on (*date*) ……...…….. 2015.

Signature of investigator Signature of witness

Declaration by translator

I *(name)* ……..…… declare that:

I assisted the investigator (*name*) ……. to explain the information in this document to (*name of participant*) ……..…….. using the language medium of Afrikaans/Xhosa/other.

We encouraged him/her to ask questions and took adequate time to answer them.
I conveyed a factually correct version of what was related to me.

I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) .....…….. on (*date*) ……...…….. 2015.

Signature of translator Signature of witness

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Approval Notice

Response to Modifications- (New Application)

23-Jan-2015
Joseph, Lauren

Ethics Reference #: S14/10/247

Title: **The ability of thromboelastogram (TEG R time difference between kaolin and heparinase) as a point of care test to predict residual heparin activity after in vitro protamine titration.**

Dear Dr Lauren Joseph,

The **Response to Modifications -** ***(New Application)*** received on **05-Jan-2015**, was reviewed by members of **Health Research Ethics Committee 2** via Expedited review procedures on **20-Jan-2015** and was approved.
Please note the following information about your approved research protocol:

Protocol Approval Period: **23-Jan-2015** -**23-Jan-2016**

Please remember to use your **protocol number** (**S14/10/247**) on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review:
Please note a template of the progress report is obtainable on www.sun.ac.za/rds and should be submitted to the Committee before the year has expired.
The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.
Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Helene Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.
For standard HREC forms and documents please visit: www.sun.ac.za/rds

If you have any questions or need further assistance, please contact the HREC office at 219389207.

Included Documents:
Response to modifications: Cover letter
Response to modifications: MPS Cert A Levin
Application form

Cover letter
Protocol
Declarations
HREC Checklist
Response to modifications: Protocol
Response to modifications: GCP Cert A Levin
CV Joseph
CV Levin
Response to modifications: MPS Cert L Joseph

Sincerely,

Mertrude Davids
HREC Coordinator
Health Research Ethics Committee 2

Chapter 3: Article

Abstract

Background: The adequate reversal of heparin with protamine at the end of cardiopulmonary bypass (CPB) is critical. Despite a difference between thromboelastographic R-kaolin time and R-heparinase time (TEG® Delta-kh R-time) indicating residual heparin, their exact relationship is not known.

Aims: We aimed to define the in-vitro relationship between (TEG® Delta-kh R-time) and plasma heparin concentrations. We also aimed to determine the concentrations of heparin at or below which R-kaolin times became measureable before a 90-minute cut-off.

Methods:

This was a single centre, prospective, randomized laboratory study. Samples were taken during CPB and randomized to a 0.25, 0.5, and 0.75 mg/mg protamine:heparin titration. This sample was subject to TEG® and heparin concentration analyses.

Results:

No relationships existed between R-kaolin times and heparin concentration (p=0.80) or between measurable TEG® Delta-kh R- time difference and heparin concentration (p=0.42). However, we did identify a high probability of being able to predict a measurable R-kaolin time (negative predictive value 90%, 95% CI 74% to 98%) with heparin concentrations < 1.24 IU/ml.

Conclusions:

We could not establish the relationship between the TEG® Delta-kh R-time difference and heparin concentrations probably due to flawed methodology. We speculated as to the reasons and suggest an alternative future methodology.

However, the study did reveal unique, useful information that R-kaolin time will likely be measurable at heparin concentrations below 1.24 IU/ml, and vice versa. This observation is immensely valuable for the clinician and also for the researcher.

Keywords: TEG, thromboelastography, heparin, cardiopulmonary bypass, protamine, bleeding, coagulopathy, activated clotting time, ACT

Introduction

Postoperative bleeding is one of the main complications after surgery involving cardiopulmonary bypass (CPB); indeed this complication occurs in approximately 20% of patients.(1). Differentiation between surgical bleeding and coagulopathy is critical. Re-exploration for post-operative bleeding is associated with an increase in mortality and morbidity (2). A surgical cause is found in 50% of patients; therefore many re-explorations can be avoided if a coagulopathy can be better identified and treated. These risks are further aggravated by the hazards, exorbitant cost and consequent intense scrutiny of the related transfusions that accompany postoperative bleeding.

It is therefore critical to monitor coagulation during and after surgery involving CPB. Laboratory tests are of little value during and after CPB, this being largely due to their delay in results. Their role in the pre-operative setting is still useful with the activated partial thromboplastin time (aPTT) being sensitive to low concentration levels of heparin. Various point of care (POC) tests such as activated clotting time (ACT), thromboelastogram (TEG®) and rotational thromboelastometry (ROTEM®) are available for this purpose. POC tests are defined as diagnostic tests at or near the bedside with the ability to produce rapid results.(3). This may provide guidance for the attending anaesthesiologist to correctly diagnose the cause of bleeding.(4). Coagulation monitoring with thromboelastography has been shown to decrease transfusion therapy and hence an improvement in outcome and diminished costs overall.(5, 6) Indeed, compared to TEG®/ROTEM® guided therapy, empiric therapy with blood and blood products are of questionable efficacy and may be hazardous to the patient.(7)

Apart from other causes of coagulopathy, it is of particular importance to monitor adequate heparinisation and conversely, heparin neutralisation with protamine. Meticulous dosing of protamine is required as excessive dosages of this drug has deleterious side effects on clotting and can aggravate bleeding. Individual responses to a dose of heparin vary. Individuals may exhibit heparin resistance and require a higher dose due to acquired or inherited antithrombin III deficiency or increased protein binding of heparin.(8). At the end of surgery, it is critical to ensure that no residual heparin remains, potentially exposing the patient to the risk of developing a

coagulopathy. This needs to be done in a timely manner and hence POC tests are ideal.

The use of POC tests has shown to reduce blood and blood product transfusion therapies. Therefore, a goal directed algorithm guides the anaesthesiologist to make informed decisions. This may alleviate the deleterious effects of transfusion therapy as well as the cost burden of such therapies.(6) Empiric or prophylactic administration of transfusion therapy during CPB should not be entertained, neither the use of an insensitive or flawed monitor. Hence the POC test chosen to make such decisions should be as accurate as possible and comparable to the gold standard.

A difference in R-time between a kaolin and heparinase TEG® implies that residual heparin is present in the blood. However, the exact relationship between the quantity of heparin that remains and the R-time difference is not known. We wanted to study the relationship between these variables.

Methods

Prior approval from the University of Stellenbosch Human Research Ethics Committee (HREC) was obtained, reference number S14/10/247. A single centre, prospective, randomized laboratory study was conducted at Tygerberg Hospital between September 2015 and December 2015. Prior informed consent was obtained from patients who were planned to undergo cardiac surgery using cardiopulmonary bypass (CPB).

The null hypothesis was that the difference between the R-times of kaolin and heparinase TEG® is not able to predict residual heparin activity after in vitro protamine titration. The alternate hypothesis was that the difference between the R-times of kaolin and heparinase TEG® is able to predict residual heparin activity after in vitro protamine titration. The primary endpoint was to determine the relationship between the difference in TEG® generated R-times (R-kaolin minus R-heparinase) and the heparin concentration. The secondary outcome was to determine the concentrations of heparin at or below which R-kaolin times became measureable, or were at least below 90 minutes.

Inclusion criteria were adults exceeding 18 years of age and 45kg body weight that were scheduled for elective ± emergent valve replacement and/or coronary artery bypass grafting (CABG) using cardiopulmonary bypass. Patients on low dose aspirin (up to 150 mg daily), even if administered on the day of surgery, as well as patients on intravenous heparin before surgery, were included. Exclusion criteria comprised patients undergoing cardiac off-pump surgery, pre-existing or suspected coagulopathy, administration of low molecular weight heparin (LMWH) in the 48 hours prior to surgery, administration of other antiplatelet agents within the two weeks prior to surgery and patients with hepatic dysfunction affecting synthesis of coagulation factors with international normalised ratio exceeding 1.5 as well as renal dysfunction with urea or creatinine exceeding 15 mmol/l and 300 umol/l respectively.

Premedication, anaesthetic techniques, monitoring, and the heparin dose were solely at the discretion of the attending anaesthesiologists.

Sampling and performance of TEG®

Each patient had three 10 ml blood samples taken from the CPB circuit, each sample being drawn with a new 20 ml plastic syringe. Protamine was added to each 10-ml blood sample. The required protamine dose was calculated using an Excel® spreadsheet that performed the following calculations:

$$EBV = BV \times weight$$

Where, EBV= estimated blood volume in ml. BV = blood volume (ml/kg) where blood volume is considered to be 65 and 75 ml/kg for females and males respectively. Weight expressed as kg.

$$P\ dose = \frac{(p{:}h)(H\ dose)(10ml)}{EBV}$$

Where, P dose is the protamine dose to be added to the 10 ml blood sample in mg, *p:h* is the protamine to heparin ratio expressed as mg/mg, and H dose is the total dose

of heparin administered to the patient (mg). Each blood sample was treated to one of three heparin: protamine ratios (0.25, 0.5, or 0.75 mg/mg) based on a randomization schedule from “www.randomization.com”. The protamine was added undiluted using an insulin syringe. After the specific protamine dose was established from the randomization schedule, ne ml of air was drawn into the 10 ml blood sample, the protamine was added and the syringe containing the protamine and blood mixture was inverted four times to ensure complete mixing. Immediately thereafter, the 10 ml blood specimen had 720 microlitres withdrawn for performance of the TEG’s® and 4.5ml thereof was added to a citrated tube for heparin activity (concentration) determination. The time frame cut-off for drawing of the blood, adding protamine, starting the TEG® and adding the sample to the citrated tube was limited to four minutes.

Quality control was performed weekly with adequate acceptable reproducibility defined as being able to conduct three sets of TEGs with less than a 10% difference between parameters. The TEG® was stopped after the R-kaolin and R-heparinase times had been established. The difference in the R-kaolin and R-heparinase time denoted for this study as TEG® Delta-kh R-time was calculated and documented. If r-times had not developed in the kaolin group before 60 minutes, the test was abandoned, but heparin activity was still determined.

Determination of Heparin levels

The citrated blood sample was placed on ice and dispatched to the NHLS within one hour of collection. The samples were centrifuged and the plasma stored at -80 degrees Celsius, at which temperature heparin is stable. Heparin assay was performed in batches using a modified anti-Xa chromogenic assay, the current gold standard method to measure heparin concentration.(9-11) A chromogenic assay, using a chromophore-linked substrate of factor-Xa cleaves the substrate by the active enzyme and subsequently releases a coloured compound. Prophylactic ranges for heparin are between 0.1 -0.4 IU/ml and therapeutic ranges are 0.3-0.7 IU/ml respectively.(10) The laboratory test incorporates known amounts of factor Xa and antithrombin III to the blood sample. The heparin forms an inhibitory complex with antithrombin III and inactivates factor Xa. The excess amount of factor Xa remaining in the sample is

inversely proportional to the original amount of heparin. The results are then compared to a standard curve and are provided in concentration of anti–factor Xa (units/mL).(10)

Sample size estimation was performed using PASS software. We assumed a linear relationship between parameters, and that the regression equation would be a straight line, i.e. $y = bx + c$, where y is the dependent variable (heparin activity) and x is the independent variable (R time difference). Using the procedure for linear regression in the PASS software, we assumed the following: that standard deviation of the x values of 0, 0.5, 1.0, 1.5 was 0.56 (i.e. using fractional values rather than percentages), a power of 0.95 was required, a two sided alpha of 0.05, a slope of at least 0.5, and a Pearson's product moment correlation coefficient (r) between x and y of at least 0.5. This revealed that a sample size of at least 42 samples would required to detect with 95% power the following relationship: A slope of 0.5 and a Pearson's product moment correlation coefficient of 0.5 with a two sided alpha value of 0.05 assuming a standard deviation of the R time differences of 56%. As this was effectively a pilot study, we increased the sample size by approximately 50% to 60 samples to ensure that we do not incur a beta error should our sample size be estimations be somewhat incorrect.

A purposely-designed form was used to collect the following clinical and laboratory data:

1. Patient demographics; patient weight, length, gender, INR, PTT, platelet count, urea, creatinine, recent anticoagulants and surgical procedure/s performed.
2. Haemoglobin concentration as measured by blood gas machine within the last 20 minutes.
3. TEG®R-kaolin (Rk) time and TEG® R-heparinase (Rh) time
4. TEG® Delta-kh R-time (i.e the difference between kaolin and heparinise TEG)
5. Heparin anti Xa assay (measured in IU/ml)

The data was entered into a Microsoft Excel® spreadsheet for processing and thereafter, statistical analysis.

Results

Statistical analysis indicated that most data were normally distributed and had equal

variance but one-way, non-parametric analysis of variance (Kruskal-Wallace ANOVA) was required to analyse the difference between the protamine:heparin 0.25, 0.5, and 0.75 mg/mg groups. This data is reported as medians (interquartile range (IQR)) with 95% confidence intervals (95% CI). Differences between the R-kaolin and R-heparinase times were analysed using a paired t-test. Pearson's or Spearman's correlation was used to determine whether relationships existed between parametric and non-parametric data respectively. Chi-squared test was used to detect differences in proportions. Logistic regression was used to identify which factor/s determined whether R-kaolin times were immeasurable and to derive a prediction probability equation. The results of the logistic regression interrogating the heparin concentration that predicted a measurable/immeasurable R-kaolin time, were subject to receiver operating curve (ROC) analysis.Twenty patients agreed to be enrolled. A total of 62 blood samples (20 in group 0.25 mg/mg, 21 in group 0.5 mg/mg, and 21 in group 0.75 mg/mg respectively) were taken. In three samples, technical errors precluded heparin concentration determination, this data being excluded from the analysis.

R-kaolin time was longer than 90 minutes in 24 TEG® analyses, precluding R-kaolin time determination in these 24 cases.

Surprisingly, Chi squared tests indicated the proportions of samples in which R-kaolin could not be determined did not differ between the Groups (Table 1). Furthermore, Kruskal-Wallace one-way analysis of variance (ANOVA) revealed no between-group differences in any of the clotting parameters measured: in other words, there were no between group differences in ACT, R-kaolin times, R-heparinase times, or heparin concentrations (Table 2).

No significant differences (p= 0.723) were identified between R-heparinase (10.4 (IQR 8.8 to 12.8) minutes) and measurable R-Kaolin times (11.2 (IQR 9.7 to 13.3) minutes) (Figure 1). Furthermore, measurable R-kaolin and R-heparinase times were strongly correlated ($r = 0.76$, $r^2 = 0.57$, $p < 0.001$) (Figure 2). Furthermore, in the 35 samples in which R-kaolin times were measurable, no relationships existed either between R-kaolin times and heparin concentrations, $p = 0.80$ (Figure 3), or between TEG® Delta-kh R- times and heparin concentrations ($p = 0.42$) (Figure 4).

Logistic regression was performed to determine whether an immeasurable R-kaolin time could be predicted. The dependent outcome (dichotomous variable) was a measurable or immeasurable R-kaolin time. The independent variables investigated whether they could predict this outcome were protamine dose, heparin activity, R-heparinase time, and ACT. Heparin concentration was the only independent variable contained within the equation, the logistic regression equation being:

$$P = \frac{1}{1 + e^{-(-2.08+1.047*hep)}}$$

Where P is the probability of the R-kaolin time being immeasurable, e is the base of the natural logarithm, and hep is heparin concentration. Heparin concentration could, in 86.6% and 50% of experiments, accurately predict that R-kaolin time would indeed be measurable and immeasurable respectively.

ROC curve analysis of the logistic regression was performed, the classification variable being measurable versus immeasurable R-kaolin time. This revealed an area under the curve (AUC) of 0.80 (95% CI 0.68 to 0.89), $p < 0.0001$, with optimum cut-off at heparin concentration greater than 1.24 IU/ml. At this value, sensitivity and specificity were 87.5% (95% CI 67.6% to 97.3%) and 71.1% (95% CI 54.1% to 84.6%), with positive and negative predictive values of 65.6% (95% CI 46.8% to 97.9%) and 90.0% (95% CI 73.5% to 97.9%), respectively (Figure 5).

Discussion

We primarily investigated if TEG® point of care testing could accurately predict residual circulating heparin concentrations. We were unable to establish a relationship between heparin concentrations and TEG® Delta-kh R-time. It was therefore not possible to validate or refute our null hypothesis. However, ROC analysis of the data indicated that R-kaolin times will be measurable at heparin concentrations below 1.24 IU/ml.

It is critical to administer heparin before initiating cardiopulmonary bypass (CPB) and properly reverse its effect after CPB is terminated. Incomplete heparin reversal and

heparin rebound following CPB occurs in as many as 50% of patients(1) and may contribute to the high incidence (20%) of post- cardiopulmonary bypass bleeding.(1) Protamine reversal of heparin is traditionally guided by the use of timeous, point of care testing rather than remote, potentially delayed, laboratory testing.(3)

ACT is a simple, convenient, widely accepted, point of care test used to indicate heparin activity. ACT is unable to measure actual heparin concentrations, but assesses the integrity of the intrinsic coagulation pathway processes. During cardiopulmonary bypass, it gauges the extent to which initial fibrin formation is affected by heparin. It is widely held that ACT increases linearly with increases in heparin concentration.(12, 13) However, the relationship between ACT and heparin concentrations is distorted by hypothermia and haemodilution,(14), thromboctyopaenia, impaired platelet function, glycoprotein IIb/IIIa inhibitors,(4, 5) and aprotinin. The Hepcon® device is a haemostatic system measuring ACT and estimating whole blood heparin concentrations using automated protamine titration. Despotis et al. demonstrated anticoagulation management during cardiopulmonary bypass with the Hepcon® device to be more reliable than the standard ACT device.(4)

Unfortunately, return of ACT to baseline is not an unqualified indicator of complete heparin reversal.(5) Indeed, research indicates that ACT is unreliable in predicting residual heparin. Galeone and colleagues described a poor relationship between plasma heparin concentrations and ACT. They concluded ACT is unable to detect residual heparin.(1) Guzetta et al investigated plasma heparin concentrations using three different ACT machines and found ACT to be an unreliable predictor of heparin concentration.(15) Effectively, ACT appears to be an insensitive point of care test to detect low residual levels of heparin, and the effects of heparin rebound. This was one of the rationales for this study.

The thromboelastogram (TEG®) is a POC test measuring whole blood viscoelastic properties allowing for more dynamic information regarding the coagulation process. Information regarding initiation and formation of the clot, the strength thereof, coagulation factor interaction and their interaction with platelets, platelet function and fibrinolysis is obtained within a short period of time. The R-time (reaction time) can be

available within 4-8 minutes and further information regarding clot kinetics within 10-20 minutes (Figures 6 to 8). The rapid availability of such useful information is appealing during and after CPB. Laboratory tests (platelet count, prothrombin time, activated partial thromboplastin time and fibrinogen levels) are not only unable to provide information on clot kinetics, but also have a much greater delay between sampling and obtaining the results.(16)

Heparin will affect the R-time alone, while other TEG® parameters are apparently unaffected by its presence.(1) An enzyme obtained from *"flaviobacterium heparinum"* called heparinase, may be added to the cuvette. Heparinase cleaves the polysaccharide portion of heparin, negating its effects and permitting the detection of non-heparin haemostatic problems.(1) If the R-kaolin time were prolonged, coagulation factor dysfunction would be a possibility. However, if R-kaolin time normalises after the addition of heparinase (R-heparinase time), the diagnosis of residual heparin can be made. Heparinase does not by itself affect TEG® variables.(17)

Galeone and colleagues demonstrated that plasma heparin concentrations correlated well with TEG® R-times, but not ACT.(1). Indeed, TEG® may be more sensitive to (even low levels of) heparin activity than other point of care or laboratory (aPTT) tests.(1, 18) Levin and colleagues compared ACT and TEG® Delta-kh R-times as endpoints of protamine titration. However, neither test proved superior.(19) The lack of a difference in sensitivity between tests was possibly because kaolin-ACT and TEG® R-kaolin times measure similar things, and that TEG® itself has a wide variability.

Limitations of TEG® include its requirements to adhere to rigorous technical standards and quality controls, the need for trained personnel, and its wide, 20% variability. Native blood testing demonstrates less variability than citrated blood.(20) Citrated blood samples are affected by sample recalcification.(20) A criticism of TEG® is that it has never undergone the validation procedures mandatory for conventional haemostatic tests such as intra- and inter-observer variability, repeatability, calibration, and quality control.(21)

In our study, we attempted to determine the relationship between the TEG® Delta-kh R-time difference and heparin concentrations. The rationale was to assess post cardiopulmonary bypass residual heparin concentrations accurately using POC testing. R-kaolin and R-heparinase times did not differ and were highly correlated. There was no relationship between heparin Concentration (Activity) (IU/ml) and TEG® Delta kh R-time. TEG® Delta-kh R- time did not predict heparin concentrations.

The question arises why a relationship between heparin concentrations and TEG® Delta-kh R-times could not be established? Baseline ACT, R-kaolin, and R-heparinase times were similar in all the groups suggesting that similar conditions existed in the groups when the blood was taken. Thus, this was not the problem. There was also no mistake in the measurement of the heparin concentrations, this being validated by the National Health Laboratory Service (NHLS) personnel. The most likely reasons lay in the complex calculations needed to calculate protamine titratation. of based on. The assumptions we made with respect to calculation of blood volumes may not have been accurate. Furthermore, we titrated miniscule amounts of protamine (typically 0.02 to 0.1 ml) using an insulin syringe, that could have introduced errors. These methodological issues will need to be overcome in similar future research. Future research would best circumvent this problem by sampling blood after incremental post cardiopulmonary bypass protamine administration. In retrospect, we originally deliberately avoided this study design as there were concerns that it would result in clumped data and prevent establishing the expected “linear” relationship. Alternatively, if the in vitro technique were reattempted, we would work at the higher concentrations of protamine and would need a larger sample size to establish a strong relationship.

Our secondary outcome, determination of at what heparin concentrations R-kaolin times would become measurable, was successful. Logistic regression was able to predict the heparin concentrations at which R-time would and would not be measurable. ROC analysis revealed a high probability (negative predictive value 90% ,95% CI 74% to 98%) of an immeasurable R-kaolin time when heparin concentrations exceeded 1.24 IU/ml. A measurable R-kaolin time was associated with a heparin concentration of less than 1.24 IU/ml, but with lesser certainty (65.6% (95% CI 46.8%

to 97.9%)). This observation can be utilised clinically. If R-kaolin time is measurable, the practitioner can deduce with reasonable certainty that that heparin concentrations are less than 1.24IU/ml.

In conclusion, we wished to establish the relationship between the TEG® Delta-kh R-time difference and heparin concentrations. Our results indicated a flawed study methodology that precluded identification of this relationship. We speculated as to the reasons for this result and suggest an alternative methodology in the future.

However, the study did reveal useful information that has to our knowledge not yet been described. R-kaolin time will likely be measurable at heparin concentrations below 1.24 IU/ml, and not measurable at concentrations above that value. This is an observation that is immensely valuable for the clinician and also for the researcher.

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Tables and Figures

	Protamine dose (mg/mg) 0.25	Protamine dose (mg/mg) 0.5	Protamine dose (mg/mg) 0.75	
R-kaolin measurable	n = 7	n = 9	n = 8	Chi-squared = 0.623 DF = 2 p = 0.723 Contingency coefficient 0.102
R-kaolin immeasurable	n = 13	n = 19	n = 12	

Table 1. The proportions of samples in which R-kaolin would be immeasurable did not differ between the groups (0.25mg/mg, 0.5 mg/mg and 0.75 mg/mg)

	Protamine dose (mg/mg)			Kruskal Wallis ANOVA
	0.25	0.5	0.75	
ACT[1] (seconds)	441.5 (420 – 467) n = 20	440.0 (413 – 461) n = 21	431.0 (417 – 461) n=21	p = 0.96
Heparin activity[2] (IU/ml)	1.03 (0.57 – 1.59) n = 20	1.42 (0.66 -2.56) n = 21	1.49 (0.73 – 2.08) n = 21	p = 0.30
R-kaolin[3] (minutes)	13.6 (8.8 – 16.4) n = 13	11.1 (9.1 – 14.1) N = 10	11.0 (9.5 – 13.9) n = 12	p = 0.80
R-heparinase[3] (minutes)	11.2 (9.5 – 13.0) n = 20	10.6 (9.3 – 13.9) n = 21	10.4 (8.7 – 12.4) n = 21	p = 0.81

Table 2. Kruskal-Wallis ANOVA revealed no between-group differences in ACT, R-kaolin time, R-heparinase time or heparin concentrations.

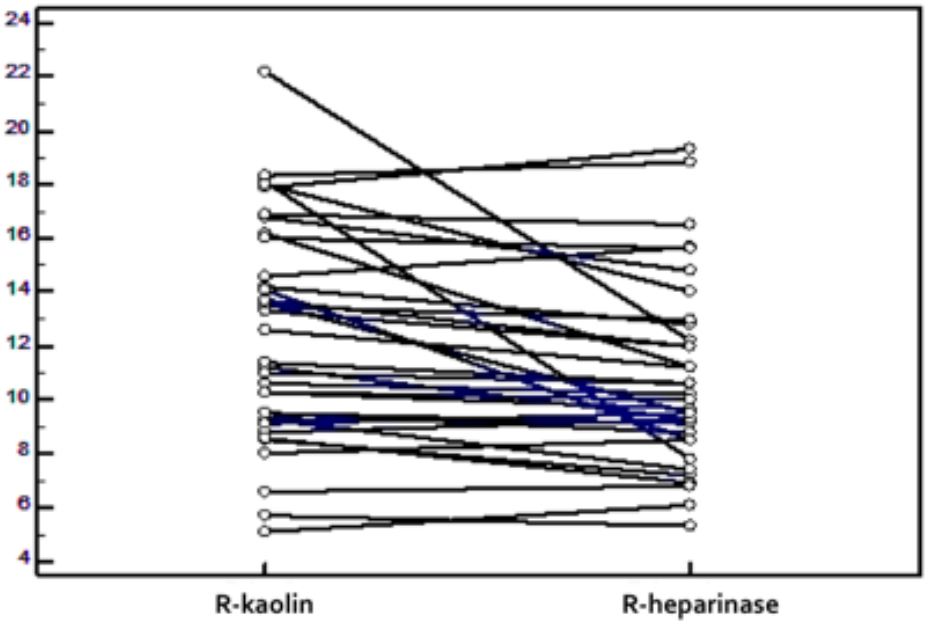

Figure 1. TEG R Kaolin and R Heparinase times in the same sample. The y axis represents R time in minutes. No significant differences were identified between R-heparinase and measurable R-Kaolin times

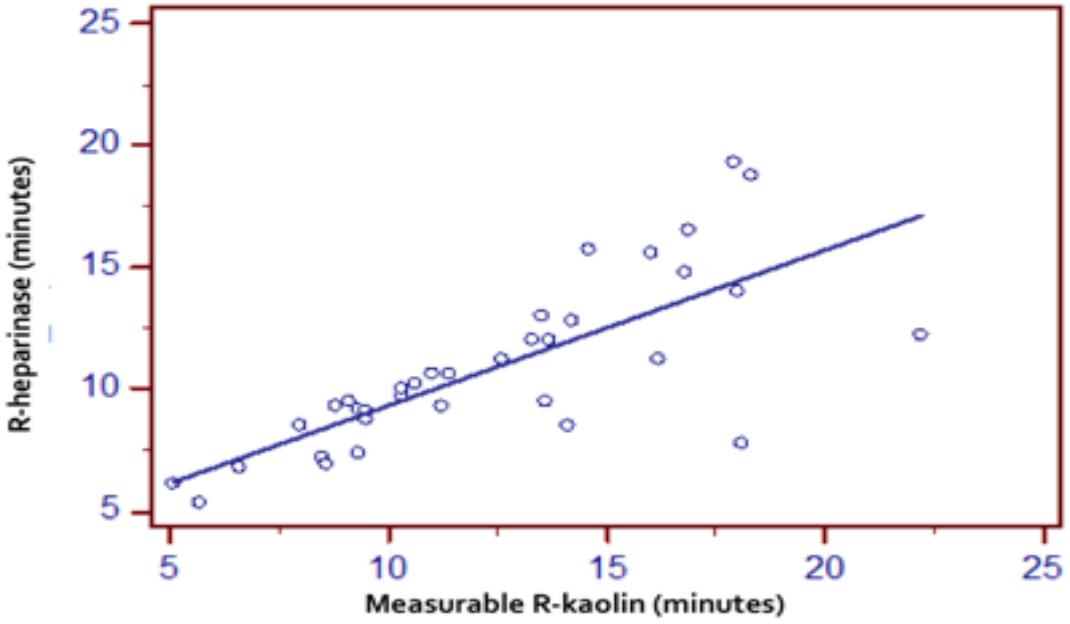

Figure 2. Strong correlation is demonstrated between measurable R-kaolin and R-heparinase times ($r = 0.76$, $r^2 = 0.57$, $p < 0.001$)

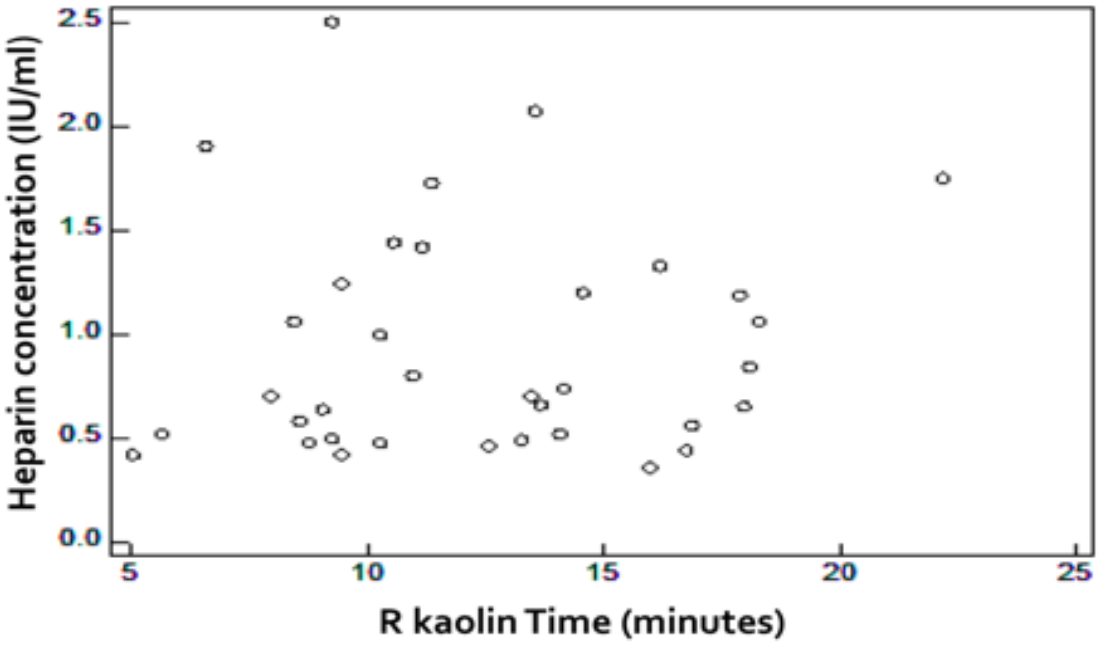

Figure 3. The scattergram of R Kaolin Time (minutes) and Heparin Concentration (Activity) (IU/ml). The R Kaolin Time could be measured in 35 instances. There is no relationship between the two variables. P=0.8

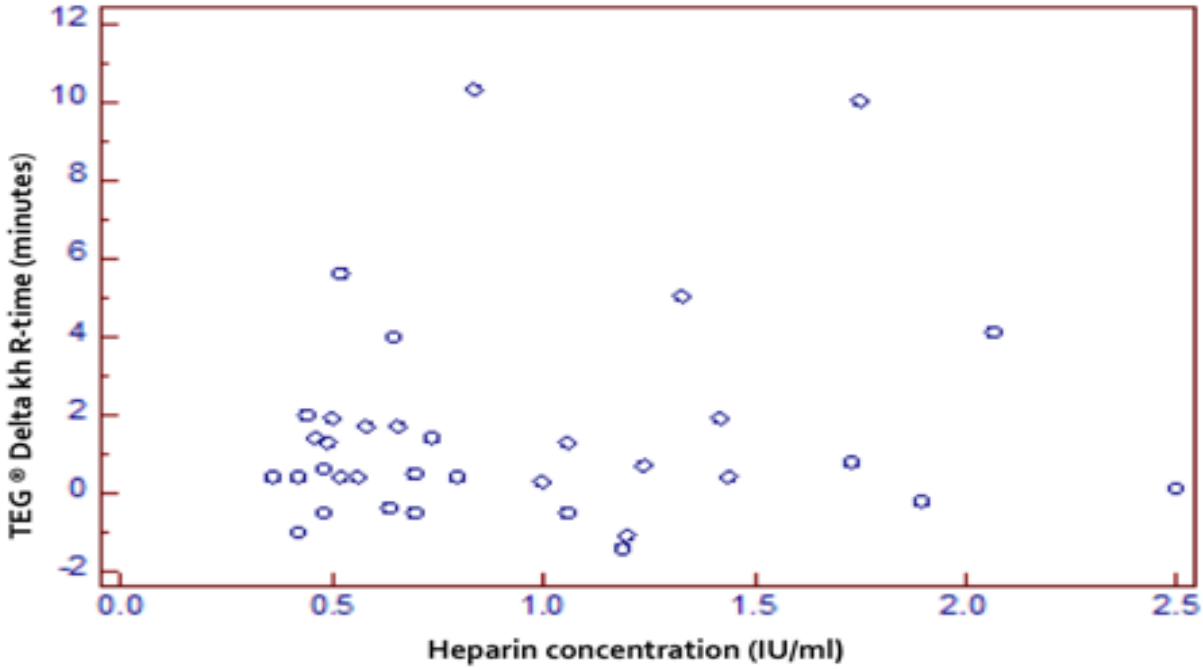

Figure 4.The scattergram of Heparin Concentration (Activity) (IU/ml) and TEG® Delta kh R-time. There is no relationship between the two variables. P=0.42

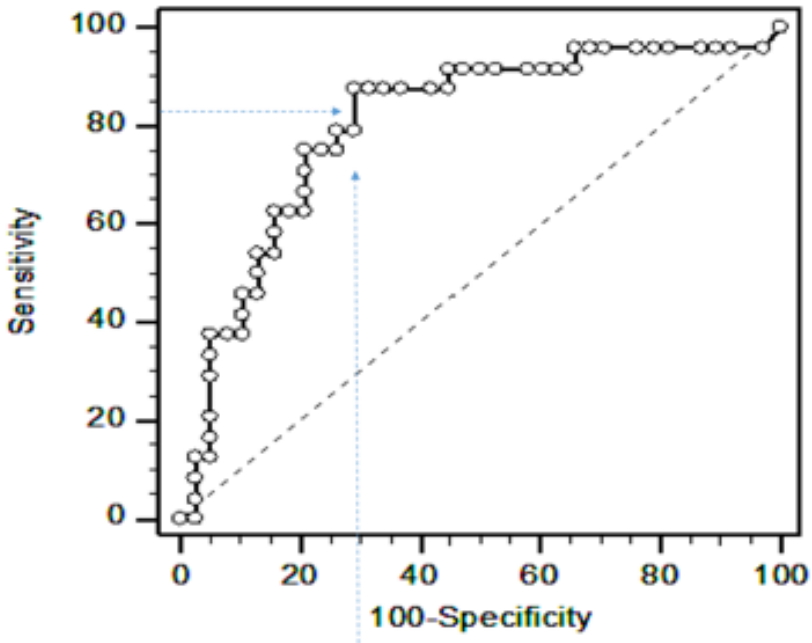

Figure 5. ROC curve analysis. There is a high probability (negative predictive value 90%) of an immeasurable R-kaolin time when heparin concentrations exceed 1.24 IU/ml. A measurable R-Kaolin time was associated with a heparin concentration of less than 1.24 IU/ml, but with lesser certainty (positive predictive value of 65.6%)

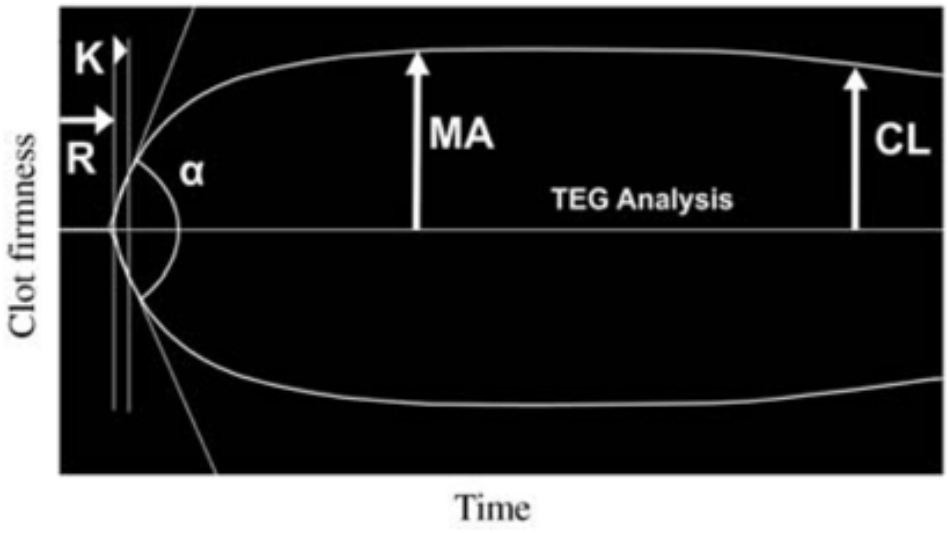

Figure 6. Thromboelastogram illustrating clot dynamics. R-time (minutes) is indicative of reaction time (initial clot formation). Heparin concentrations only affect the R-time

Parameter	Units	Definition	TEG®
Clotting time	s	Period from 0 to 2mm amplitude	Reaction Time R
Clot kinetics	s	Period from 2 to 22mm amplitude Slope between r and k/slope of tangent at 2mm amplitude	Kinetics K Time
Clot strengthening	deg		α
Amplitude **Maximum strength** **Lysis**	mm	Maximal amplitude	A Maximum amplitude MA CL 30, CL 60

Figure 7. TEG® variables

Test	Activator/inhibitor	User/indication
Native	None	Non-activated assay
Kaolin	Kaolin	General coagulation assessment including platelet function
Heparinase	Kaolin and heparinase	Detection of heparin
Platelet mapping	Adenosine diphosphate arachidonic acid	Platelet function monitoring during anti-platelet therapy

Figure 8. Commercially available TEG® assays